1219 Vascular Dysfunction: Mechanisms

Wednesday, April 1, 1998, 3:00 p.m.–5:00 p.m.
Georgia World Congress Center, West Exhibit Hall Level
Presentation Hour: 3:00 p.m.–4:00 p.m.

1219-1 Hypothermia Induces Nitric Oxide Release From the Arterial Endothelium: Mechanism of Early Hypotension Following Institution of Cardiopulmonary Bypass

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Background: The onset of hypothermic cardiopulmonary bypass is initially associated with a decrease in peripheral resistance resulting in hypotension. Nitric oxide is an endogenous vasodilator produced by the arterial endothelium. Experiments were undertaken to determine if hypothermia induces the release of endothelium-derived nitric oxide (EDNO) from arterial conduits.

Methods and Results: Segments of contracted (prostaglandin $F_{2\alpha}$, 2 × 10^{-6}M) canine coronary, femoral and renal artery, with and without endothelium, were exposed to progressive hypothermia (from 37 to 20°C). Hypothermia induced vasodilation of arterial segments with endothelium (to 93 ± 4% of the initial contraction for femoral artery segments, means ± SEM, n = 5, each group, $P < 0.05$). In all groups, endothelium-dependent vasodilation to hypothermia was blocked by L-NMMA or NO-ARG (10^{-5}M), two competitive inhibitors of nitric oxide synthesis from L-arginine (n = 5, each group, $P < 0.05$). Vasodilation was also inhibited by hemoglobin (2×10^{-6}M) (n = 6, $P < 0.05$). Vasodilation to hypothermia was completely inhibited by the addition of atropine or pirenzepine (10^{-6}M) (n = 5, each group, $P < 0.05$).

Conclusions: Endothelium-dependent vasodilation to hypothermia in systemic and coronary arteries, presumably mediated by the M_1 muscarinic receptor, could be the mechanism for the decrease in peripheral vascular resistance and hypotension associated with the onset of hypothermic cardiopulmonary bypass.

1219-2 Adrenomedullin Induces Vasodilation in Porcine Coronary Conductance and Resistance Arteries In Vivo

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Background: Adrenomedullin (ADM) is a newly discovered vasodilator peptide found in the heart, lung, adrenals and vascular endothelium. Its physiological role is unknown. Although vasoactive effects have been described, its role in the coronary circulation is unclear.

Objectives: We examined the acute vasodilator effect of intracoronary (IC) ADM on epicardial and resistance coronary arteries in 15 pigs.

Methods: Epicardial coronary cross-sectional area (CSA) and average peak flow velocity (APV) were assessed using simultaneous intracoronary two-dimensional and Doppler ultrasound. Coronary blood flow (CBF) was calculated. Amplification by RT-PCR was performed to demonstrate the expression of the ADM receptor gene.

Results: ADM (0.01nM to 0.1 μM/L IC) induced a significant increase in coronary CSA (8.93 ± 1.23%), APV (16.63 ± 4.67%) and CBF (26.65 ± 4.62%). Following preconstriction with endothelin-1 (10 nM/L IC), there was no increase in ADM-induced vasodilation. Pretreatment with "nitro-L-arginine methylester (100 μ/L IC) significantly attenuated ADM-induced increase in CSA ($P < 0.0001$), APV ($P = 0.0046$) and in CBF ($P = 0.0001$). By RT-PCR, the intensity of the ADM receptor gene signal was greater in epicardial coronary arteries than in lung tissue (used as control).

Conclusions: ADM induces vasodilation in coronary conductance and resistance arteries in pigs, in part mediated via nitric oxide. ADM receptor gene is expressed in coronary conductance arteries.

1219-3 Short-term Exposure to Second Hand Smoke Induces Vascular Dysfunction in Normocholesterolemic Rabbits

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Background: Second hand smoke (SHS) contributes to about 37,000 out of a total 53,000 heart disease deaths in the US. We have previously shown that chronic (10 weeks) SHS exposure causes endothelial dysfunction in normocholesterolemic rabbits. We hypothesized that short-term exposure to SHS would also cause endothelial dysfunction.

Methods: Ninety six normocholesterolemic rabbits (16/group) were exposed to SHS in smoking chambers (4 cigarettes/15 minutes) for 0 (control), 1/2, 1, 2, 4 and 6 hours. Vascular reactivity was examined in vitro in aortic rings. Vasoconstriction was assessed in response to the alpha$_1$-adrenoceptor agonist phenylephrine (Phe). Following preconstriction with the EC50 dose of Phe, vasorelaxation responses were assessed using the endothelium-dependent vasodilator acetylcholine (Ach) and the endothelium-independent vasodilator nitroglycerin (Ntg).

Results: Short-term SHS exposure caused a decrease in maximal response and sensitivity to Phe, but an increase in the rate of vasoconstriction (slope $p = 0.001$). Following 1/2 and 1 hours of SHS exposure there was a trend towards depressed maximal endothelium-dependent relaxation with Ach, that was significant at 2 hours (66% ± 4 Vs 71% ± 4 at 0 hrs, $p < 0.05$). However, at 4 and 6 hours Ach-induced vasorelaxation appeared to be restored. Short-term SHS exposure caused no change to Ntg-induced vasodilation.

Conclusions: Short-term exposure to SHS influences adrenoceptor-mediated vasoconstriction and causes transient endothelial dysfunction in normocholesterolemic rabbits.

1219-4 Effect of Passive Smoking on Arterial Wave Reflection: An Additional Detrimental Effect

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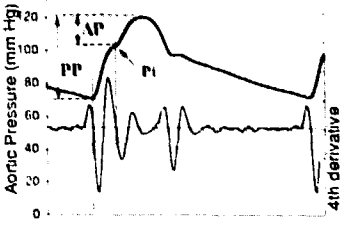
Background: We have previously shown that active smoking deteriorates function the human aorta (Circulation 1997;95:31-38). However, wave reflection along the arterial bed, an important index of arterial stiffening and cardiac afterload, has not been studied during passive smoking.

Methods: High-fidelity pressure waveforms of the thoracic aorta were obtained before and for 20 min. after the initiation of passive smoking (exposure for 5 min, CO: 30 p.p.m. 10 pts), or sham smoking in 10 pts with an intravascular catheter-tip micromanometer (Millar Instr.) during diagnostic catheterization. In all pts, an inflection point (Pi) defined by computer algorithm (4th derivative) divided aortic pressure waveform into early and late systolic phase (fig). Augmentation index was defined as: AP/PP (fig).



Results: Both systolic and diastolic pressures increased with passive smoking (peak at min. 4, from 126 ± 14 to 137 ± 12 and from 74 ± 8 to 80 ± 7 mmHg, respectively, $P < 0.001$). Augmentation index increased with passive smoking (from 0.29 ± 0.12 to 0.33 ± 0.14, $P < 0.05$, peak: min. 4) indicating increased wave reflection in the periphery.

In contrast, no changes were observed with sham smoking.

Conclusions: Wave reflection is increased with passive smoking. This effect may contribute in the multiple adverse consequences of passive exposure to tobacco smoke in the cardiovascular system.

1219-5 Raman Spectroscopy Provides Chemical Mappings of Atherosclerotic Plaques in APOE*3 Leiden Transgenic Mice

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Background: The chemical composition of the arterial wall may change during plaque development and determines whether a plaque will progress, regress or rupture. APOE*3 Leiden transgenic mice, which overexpress a dysfunctional human apolipoprotein E variant, develop hyperlipidemia and diet-induced atherosclerotic plaques, similar to those in humans. To map the chemical composition of atherosclerotic plaques we used near-infrared Raman spectroscopy.

Methods: APOE*3 Leiden transgenic mice (n = 14) were fed either a high saturated-fat/high-cholesterol/0.5%-cholate (HFC/0.5%-cholate) diet or normal chow for 5 or 6 months. The mice were sacrificed, and their aortas (~3.5 mm in circumference) were flushed and cut open for spectroscopic examination. Raman spectra were obtained from locations across the luminal surface

to create a grid of measurements at 0.5 mm spacing. The spectra at each location were processed to calculate the weights of cholesterol and calcium salts relative to the dehydrated weight of the aorta in a spectroscopically examined volume.

Results: In the aortic wall of mice fed normal chow, cholesterol and calcium salts each accounted for < 3% of the relative weight at all examined locations. Mice that received the HFC/0.5%-cholate diet developed cholesterol-rich plaques in a highly reproducible fashion. The plaques had calcified cores in the center of these plaques, all located just distal to the aortic valve at the inner curvature of the arch. Cholesterol and calcium salts amounts reached maximum values up to 61 and 73%, respectively.

Conclusion: Raman spectroscopy can map the chemical composition of atherosclerotic plaques from transgenic mice, which could aid the study of atherogenesis. This technique may help to assess the efficacy of hypolipidemic and anti-atherosclerotic therapy.

1220 Basic Studies of Myocardium

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1220-25 Detection of Microsatellite Alterations in Sporadic Cardiac Myxomas

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Background: Microsatellite instability (MI) is an early event in DNA repair deficient associated diseases and reflects an elevated mutation rate in the genome of neoplastic cells. Furthermore, loss of heterozygosity (LOH) reflects the inactivation of tumor suppressor genes which in turn provide information for the molecular pathway of human oncogenesis. This study investigates the incidence of microsatellite alterations (MI and LOH) in sporadic cardiac myxomas.

Methods: Eleven surgically excised sporadic cardiac myxomas were assessed for MI and LOH in different chromosomes (2, 3, 6, 7, 8, 9, 11, 17, 18, 19). DNA was extracted from myxoma tissue specimens as well as the respective normal myocardial tissue and subjected to polymerase chain reaction (PCR) with 22 highly polymorphic microsatellite markers. PCR products were electrophoresed in a 10% polyacrylamide gel and silver stained. Microsatellite alterations were scored by comparing the electrophoretic pattern of the microsatellite markers amplified from the paired DNA preparations that corresponded to the myxoma tissue with adjacent normal tissue. The analysis in positive cases was repeated at least twice and the results were highly reproducible.

Results: The microsatellite analysis revealed that six myxoma specimens (54%) exhibited MI in at least one marker. One myxoma specimen exhibited evidence of MI in three markers, while the marker with the most frequent incidence of MI was D17s855 (25%), which lies within BRCA 1. Furthermore, chromosome 17 demonstrated more frequently MI than the others, especially within and near the location of BRCA 1. Finally, no LOH was observed in myxoma specimens.

Conclusions: We have detected a considerable incidence of MI in sporadic cardiac myxomas indicating that decreased fidelity in DNA replication and repair is common in myxoma tissue. To the best of our knowledge, this is the first report describing MI in sporadic cardiac myxomas, as a possible pathogenetic mechanism of these rare neoplasms.

1220-26 Enalapril Selectively Improves Smooth Muscle Myosin Heavy Chain Isoform Expression in Intramyocardial Arteries of Spontaneously Hypertensive Rats

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Background: Phenotypic modulation of smooth muscle (SM) myosin heavy chain (MHC) isoforms in intramyocardial arteries (IMA) may play a role in coronary flow reserve (CFR) in hypertension and might be affected by angiotensin II.

Method: We compared the effects of AT1 blocker, FK-739 (FK;30 mg/kg/day), enalapril (EN;10 mg/kg/day) and vehicle on phenotipic modulation of SM-MHC isoforms (SM1, SM2, and NMHC-B) in IMA in 14-week-old spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY) for 6 weeks.

Results: Body weight and heart rate were not different among 6 groups. Left ventricular (LV) systolic pressure was higher in vehicle SHR than the other groups ($p < 0.001$), and also higher in both FK and EN-treated SHR groups than WKY groups ($p < 0.01$). In SHR, LV weight/body weight ratio, perivascular fibrosis and wall thickness/lumen ratio were higher in vehicle SHR than EN and FK groups ($p < 0.01$), but no difference was observed in FK and EN-treated SHRs. These effects of EN and FK were not observed in WKY groups. Immunohistochemically, SM1, SM2 and NMHC-B were selectively expressed in SM cells of IMA. Immunoblot showed that SM1 was less expressed in vehicle SHR than WKY groups ($p < 0.005$), and EN treatment increased in SM1 expression in SHR ($p < 0.05$). In contrast, SM2 and NMHC-B were unchanged by EN and FK in both SHR and WKY groups. Cardiac angiotensin II was higher in SHR compared with WKY ($p < 0.01$) and significantly reduced by EN ($p < 0.01$), but not by FK.

Conclusion: SM1 in IMA was decreased in SHR compared with WKY. A 6-week-treatment of EN selectively improved SM1 expression but not FK, suggesting that EN may have more potent effect on contractile properties of SM in IMA of SHR.

1220-27 L-Arginine Decreases Blood Pressure and Left Ventricular Hypertrophy in Rats With Experimental Aortic Coarctation

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Dietary supplementation of L-Arginine, the substrate for nitric oxide synthase, has been shown to decrease atherosclerosis and ameliorate endothelial function in hypercholesterolemic rabbits. The benefits of L-Arginine in hypertension are less clear. To evaluate the effect of L-Arginine on blood pressure and left ventricular hypertrophy, we administered L-arginine (2.25% in drinking water) to rats in which blood pressure was increased by suprarenal abdominal aortic banding. 34 Sprague-Dawley female rats (230–260 g) were randomized to sham-operated (S), untreated (C) and arginine-treated (A) groups for 7 weeks. We measured heart rate, systolic and diastolic arterial (SBP, DBP) and left ventricular pressure in mmHg (SP-LV, DP-LV). Left ventricular hypertrophy was assessed as the ratio of LV weight to body weight (LV/BW) and LV wall thickness (LV-T, mm). Additionally, aortic rings were harvested, and vascular reactivity in response to acetylcholine and nitroglycerin were assessed in vitro in organ baths. M ± SEM, * $p < = 0.001$, # $p = 0.028$

Group	SBP*	DBP*	DP-LV#	LV-T*
S (11)	101 ± 2	72 ± 2	0 ± 0	3.33 ± 0.08
C (11)	124 ± 4	92 ± 4	14 ± 5	4.11 ± 0.07
A (12)	107 ± 4	76 ± 3	9 ± 3	3.31 ± 0.05

L-arginine significantly attenuated the increases in SBP and DBP (both $p = 0.003$) and in wall thickness of LV ($p < 0.001$) by 2-way ANOVA. However, L-arginine had no significant effects on either the increase in LV/BW or the vasorelaxation responses to acetylcholine and nitroglycerin. We conclude that dietary L-arginine supplementation decreases blood pressure and wall thickness in experimental hypertension, without a significant improvement in vascular endothelial function. The use of L-Arginine in clinical hypertension merits further study.

1220-28 Comparative Time Delays of QT Interval Adjustement to Heart Rate During Autonomic Blockade and After Fixed Rate Atrial Pacing in Young Normal Subjects

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Background: QT interval adjustment to changes in heart rate seems to be time-dependent, a phenomenon which is not readily visible on standard ECG tracings but can be assessed by imposing a steady cardiac rhythm.

Methods: After informed consent, 12 males aged 19–20, evaluated for various reasons and proven to be healthy, underwent autonomic blockade (AB) and right atrial pacing (RAP) in 2 separate days. Both interventions were done under continuous recording of a bipolar thoracic ECG lead chosen to have positive R waves and maximal T wave amplitude for beat-by-beat RR and QT interval measurement with a resolution of 1 ms (Codas, Dataq Instruments, USA). AB consisted of i.v. administration of propranolol 0.2 mg/kg in 15 min followed by atropine 0.04 mg/kg in 2 min. RAP was performed for 7 min at 2 fixed rates (the slowest with stable atrial capture as referral level and 100 beats/min) with an abrupt switch to the second pacing rate. Time intervals were measured by computer and data were compared using the Mann-Whitney-Wilcoxon test.

Results: After AB both RR and QT intervals decreased exponentially and became stable with time constants of (mean ± SD) 29.8 ± 14.4 s (τ_{RR-AB})